



GLUT12 functions as a basal and insulin-independent glucose transporter in the heart

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ABSTRACT

Glucose uptake from the bloodstream is the rate-limiting step in whole body glucose utilization, and is regulated by a family of membrane proteins called glucose transporters (GLUTs). Although GLUT4 is the predominant isoform in insulin-sensitive tissues, there is recent evidence that GLUT12 could be a novel second insulin-sensitive GLUT. However, its physiological role in the heart is not elucidated and the regulation of insulin-stimulated myocardial GLUT12 translocation is unknown. In addition, the role of GLUT12 has not been investigated in the diabetic myocardium. Thus, we hypothesized that, as for GLUT4, insulin regulates GLUT12 translocation to the myocardial cell surface, which is impaired during diabetes. Active cell surface GLUT (-4 and -12) content was quantified (before and after insulin stimulation) by a biotinylated photolabeled assay in both intact perfused myocardium and isolated cardiac myocytes of healthy and type 1 diabetic rodents. GLUT localization was confirmed by immunofluorescent confocal microscopy, and total GLUT protein expression was measured by Western blotting. Insulin stimulation increased translocation of GLUT-4, but not -12, in the healthy myocardium. Total GLUT4 content of the heart was decreased during diabetes, while there was no difference in total GLUT12. Active cell surface GLUT12 content was increased in the diabetic myocardium, potentially as a compensatory mechanism for the observed downregulation of GLUT4. Collectively, our data suggest that, in contrast to GLUT4, insulin does not mediate GLUT12 translocation, which may function as a basal GLUT located primarily at the cell surface in the myocardium.

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1. Introduction

The heart is one of the main organs to utilize glucose as an energy substrate in order to provide a constant ATP supply to support the high rate of cardiac power. Glucose transport and utilization by cardiac myocytes is critical for maintenance of cardiac function. As a consequence, diabetes, which is characterized by dysfunctional glucose uptake into striated muscles, leads to major impairments in both myocardial metabolism and function [1–3]. Glucose uptake from the bloodstream is the rate-limiting step in whole body glucose utilization, and is regulated by a family of membrane proteins called glucose transporters (GLUTs). The well-established GLUT4 is the predominant isoform in the heart and provides the majority of insulin-stimulated glucose uptake by a translocation process from an intracellular (non active) pool to the plasma membrane (active site). Although it has been thought that GLUT4 translocation is the rate-limiting step for

glucose uptake and utilization [4], GLUT4 knockout mice do not develop hyperglycemia, suggesting that other GLUT isoforms may also be involved in the regulation of whole body glucose homeostasis [5]. Indeed, thirteen other isoforms have been identified, and divided into 3 different classes based on their distinct regulatory and/or kinetic properties that reflect their specific roles in cellular and whole body glucose homeostasis [6]. Importantly, there is recent evidence that GLUT12 (member of Class III) could be a novel second insulin-sensitive GLUT in skeletal muscle [7,8]. Furthermore, GLUT12 mRNA is highly expressed in adult human hearts [9] and GLUT12 protein is present in significant quantities in insulin-sensitive tissue, including the heart [10]. Clearly, the potentially key role for GLUT12 in the regulation of myocardial glucose homeostasis warrants timely investigation. However, insulin stimulation of GLUT12 translocation has not been investigated in the heart and its contribution to normal cardiac glucose transport is unknown. Furthermore, while a recent study has demonstrated a significant role for GLUT12 in regulating whole body insulin sensitivity [8], its function in the pathogenesis of insulin resistance and diabetes has not been well established, and the role of GLUT12 in the diabetic myocardium has not been studied. Better understanding of the role of this most recently identified GLUT isoform could provide novel insights into the regulation of myocardial glucose transport during physiological and pathological conditions, which

Abbreviations: GLUT, glucose transporters; Dx, diabetes; Con, control; L, labeled; UL, unlabeled; CASQ, calsequestrin

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could lead to the identification of novel pharmacological targets. Therefore, we hypothesized that, as for GLUT4, insulin regulates GLUT12 translocation to the cell surface in the healthy myocardium, which is impaired during diabetes.

2. Methods

2.1. Animals

Healthy and insulin-deficient diabetic (Dx) FVB/N mice were used. Type 1 diabetes was induced at 12–16 weeks of age by 3 consecutive streptozotocin injections (65–95 mg/kg IP every 48 h). The control group received similar volume of vehicle. Venous blood was drawn weekly from the facial vein for measurement of glucose, using a glucometer (Bayer Contour, Tarrytown, NY) on mice previously fasted overnight for 12 h. In vitro experiments were performed at 8 weeks after the induction of diabetes or in age-matched healthy controls, 24–48 h after the final blood [glucose] sample was taken. All procedures were approved by the Ohio State University Institutional Animal Care and Use Committee.

2.2. Western immunoblotting

Crude extracts of membrane-enriched ventricular myocardium or gastrocnemius skeletal muscle were prepared as previously described [10–13]. Briefly, frozen tissue samples were homogenized in buffer, which contains (mM): sucrose 210, NaCl 40, EDTA 2, HEPES 30, and protease inhibitor (Sigma, St. Louis, MO). The homogenate was incubated with sodium pyrophosphate 58 mM and KCl 1.17 mM. Crude membranes were then recovered by centrifugation at 100,000 g for 90 min at 4 °C. Pellets were resuspended with a solution containing (mM): Tris·HCl 10, and EDTA 1. After addition of SDS (16%, 0.33 total volume), samples were centrifuged at 3000 g for 25 min, and the supernatant was stored at –80 °C until Western blot analysis. Extracts were subsequently resolved by sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis, electrophoretically transferred to PVDF membranes, and subjected to immunoblotting [10–13]. Membrane proteins were incubated with rabbit polyclonal antibodies against human GLUT4 (1:7500, AbD Serotec, Raleigh, NC) or against rat GLUT12 (1:500, Abcam, Cambridge, MA). Primary antibodies were chosen based on their 100% sequence homology with the protein in mice, and validated against a positive control. Subsequently, the membrane was incubated with secondary antibody conjugated to horseradish peroxidase (GE Healthcare, Buckinghamshire, UK). Quantitative determination of protein was performed by autoradiography after revealing the antibody-bound protein by enhanced chemiluminescence reaction. Equal protein loading was confirmed by reprobing each membrane for calnexin protein expression (1:2500, Millipore, Billerica, MA).

2.3. GLUT translocation to the cell surface

Isolated hearts underwent a 30-min baseline Langendorff perfusion followed by 35 min of additional perfusion with or without insulin (at physiologic concentration, 100 µU/ml) before photolabeling with the cell surface impermeant biotinylated bis-mannose reagent (bio-LC-ATB-BGPA, 300 µM), of which the hexose group interacts specifically with the extracellular binding site of GLUT [14,15]. The photolabeled reagent was infused through the aorta and photochemically cross-linked to cell surface GLUTs using a Rayonet photochemical reactor (340 nm, Southern New England UV). The heart was then flash-frozen in liquid nitrogen and stored at –80° until further analysis. Total cardiac membrane extracts of photolabeled myocardial tissue (“total lysate” fraction) were incubated with streptavidin bound to 6% agarose beads (Pierce, Rockford, IL), which allows separation of non-cell surface GLUTs (“unlabeled” or intracellular fraction) that

remain in the supernatant from cell surface GLUTs (“labeled” or sarcolemmal fraction) [11,15]. All fractions were then subjected to SDS-PAGE and subsequent immunoblotting with GLUT-4 or -12 antibody as described above. In a separate set of experiments, cardiac myocytes were isolated by perfusion in a retrograde manner using a Langendorff apparatus [16], and then incubated for 20 min without (basal) or with insulin (100 µU/ml). Bio-LC-ATB-BGPA photolabeled compound was then added to the incubation media and cross-linked to cell surface GLUTs after 1 min. The cells were then washed with Tyrode, solubilized in lysis buffer, and subjected to protein quantification and Western blotting as described above.

2.4. Immunofluorescent confocal microscopy

The effect of insulin on the subcellular distribution of GLUT4 and GLUT12 was examined by confocal laser scanning microscopy, as described previously [17]. In brief, cardiac myocytes were incubated without or with insulin (100 µU/ml) for 30 min prior to immunofluorescent staining. Cells were fixed with 4% formaldehyde in PBS for 30 min, blocked, permeabilized in 5% goat serum in PBS with 0.1% Triton X-100 (30 min), and labeled with primary antibody for overnight. Cells were then washed three times and labeled with fluorescence-conjugated secondary antibody for 1 h. Immunofluorescence was visualized with confocal laser scanning microscopy (Olympus FV 1000). All images were analyzed offline.

2.5. Statistics

Differences between means were assessed by paired *t*-test, Student's *t*-test or ANOVA, as appropriate (Sigmatat, Jandel Scientific). When a significant difference was identified by ANOVA, post hoc tests were performed using the Student–Newman–Keuls technique. Statistical significance was defined as $P < 0.05$. Data are presented as mean ± SE.

3. Results

3.1. Basal cardiac GLUT regulation

Since the regulation of GLUT12 in the heart has not been investigated in healthy and diabetic state, we first measured GLUT protein expression from a crude membrane-enriched fraction in cardiac muscle. Compared to healthy state, cardiac crude membrane GLUT4 content was decreased in the diabetic heart, while there was no change in crude membrane GLUT12 (Fig. 1). By contrast, insulin-dependent diabetes resulted in a significant increase in GLUT12 protein content in skeletal muscle (Fig. 1S). However, it is important to note that the crude membrane extract contains total cellular membranes, and thus active plasma membrane-associated GLUTs cannot be accurately differentiated from inactive GLUTs that are associated with intracellular membranes or that have not yet fused with the plasma membrane [15]. Therefore, we used a biotinylation assay in the intact perfused heart to reliably and specifically quantify the proportion of active GLUT at the myocardial cell surface (Fig. 2). As expected, diabetes decreased basal active cell surface GLUT4 content; by contrast, basal cell surface GLUT12 content was increased during diabetes (Fig. 2A). Furthermore, we quantified the proportion of GLUT located at the myocardial cell surface compared to intracellularly for both GLUT-4 and -12 in the photolabeled myocardium. As expected, under basal condition, the amount of GLUT4 at the cell surface was 31.1 ± 6.1 and $18.6 \pm 6.77\%$ of total myocardial GLUT4 protein in healthy and diabetic myocardium, respectively (Fig. 2B). By contrast, basal cell surface GLUT12 content contributed to the majority of myocardial GLUT12 protein, such that it was 64.4 ± 17.3 and $63.2 \pm 9.04\%$ of total myocardial GLUT12 protein in healthy and diabetic myocardium, respectively (Fig. 2C).

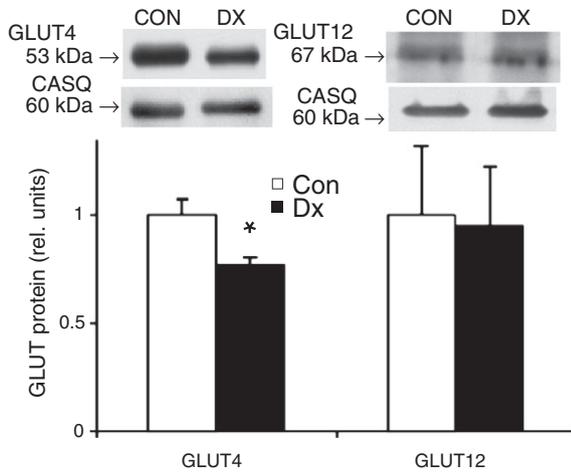


Fig. 1. In a crude membrane-enriched fraction, insulin-dependent diabetes results in no change in cardiac GLUT12 protein content, despite decreased cardiac GLUT4. Top panel: Western blot of cardiac muscle from healthy (Con) and type 1 diabetic (Dx) mice. Total calsequestrin (CASQ) protein content was used as a loading control. Bottom panel: Mean \pm SE total GLUT protein content in a crude membrane-enriched fraction, (n = 5–10/group), *P < 0.05 vs. control group.

3.2. Insulin-stimulated cardiac GLUT regulation

We then investigated whether as for GLUT4, cardiac GLUT12 is regulated by insulin stimulation using the biotinylation assay in healthy cardiac myocytes incubated without (basal) or with insulin. As expected, physiologic insulin stimulation resulted in increased active cell surface GLUT4 content compared to basal unstimulated healthy myocytes, while there was no difference in cell surface GLUT12 compared to basal condition (Fig. 3A). To further confirm the lack of regulation of GLUT12 by insulin, we also performed the biotinylation assay in the isolated heart perfused with or without insulin (Fig. 3B). Similarly, physiologic insulin stimulation increased active cell surface GLUT-4, but not -12, content in the healthy myocardium (Fig. 3B). We further observed that diabetes impaired insulin-stimulated GLUT4 translocation to the cell surface, while there was no difference in cell surface GLUT12 compared to basal condition in the diabetic myocardium (Fig. 3A). These results using the progressive biotinylation methodology were also further confirmed by immunofluorescent confocal microscopy such that, while both GLUT4 (Fig. 4A) and GLUT12 (Fig. 4C) were associated with cytosol, peripherals and T-tubular-like structures in healthy, noninsulin-stimulated myocytes, physiologic insulin stimulation caused significant translocation of GLUT4 (Fig. 4B), but not GLUT12 (Fig. 4D) to cell peripherals.

3.3. Cardiac GLUTs and whole-body glucose homeostasis

We then investigated the potential contribution of GLUT-4 and -12 to whole-body glucose homeostasis under physiological and pathological conditions. As expected, our model of streptozotocin-induced type 1 diabetes resulted in sustained hyperglycemia from 1 week after injection until the end of the study (Fig. 5A). Linear regression analysis of active basal cell surface GLUT content (independent variable) in the isolated perfused heart and venous blood [glucose] (dependent variable) shows a significant negative correlation between active cell surface GLUT4 and blood [glucose] in the healthy and diabetic myocardium ($P = 0.007$, $R_{sq} = 0.463$; Fig. 5B). Conversely, there was a significant positive correlation between active cell surface GLUT12 and blood [glucose] in these mice ($P = 0.045$, $R_{sq} = 0.317$; Fig. 5C). There was no significant correlation between active cell surface GLUT-4 and -12 ($P = 0.62$, $R_{sq} = 0.018$, data not shown).

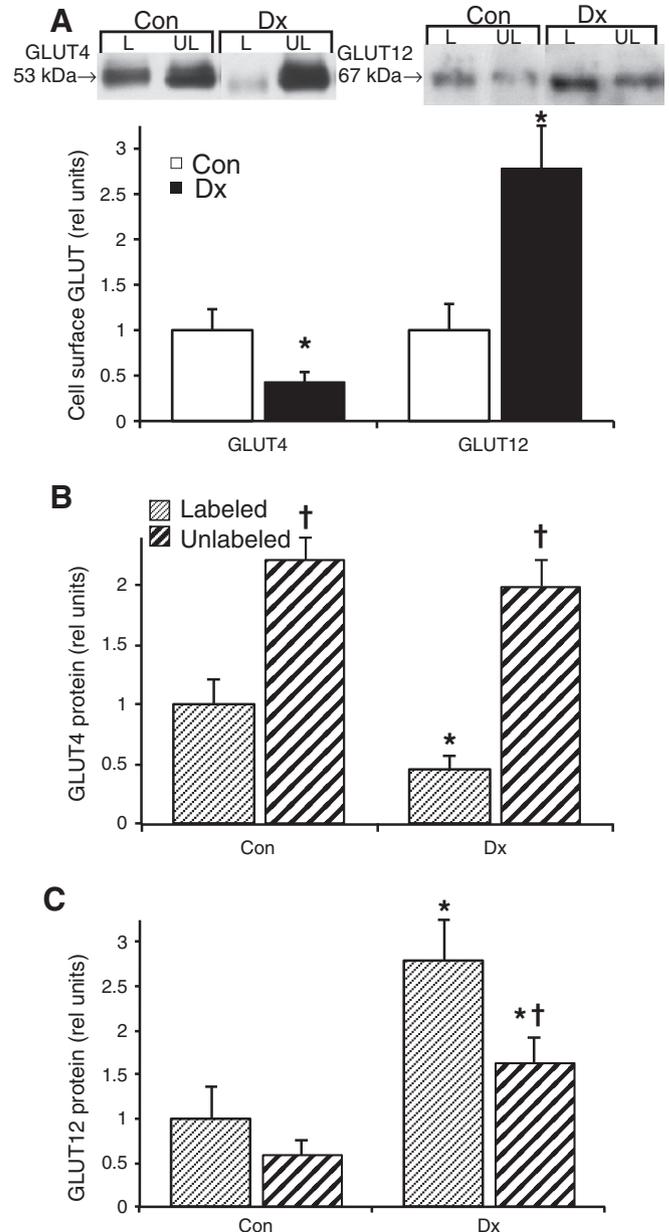


Fig. 2. A) Diabetes increases active cell surface GLUT12 in photolabeled intact perfused myocardium. Isolated perfused hearts from healthy (Con) and type 1 diabetic (Dx) mice were photolabeled with bio-LC-ATB-BGPA to determine the amount of cell surface (L: labeled fraction) and intracellular (UL: unlabeled fraction) content. Top panel: Representative Western Blot. Bottom panel: Mean \pm SE GLUT protein content under basal conditions (expressed relative to internal control; n = 7–10/group). B) While GLUT4 is primarily located intracellularly, C) the majority of GLUT12 protein is active at the cell surface. Method: Isolated perfused hearts from healthy (Con) and type 1 diabetic (Dx) mice were photolabeled with bio-LC-ATB-BGPA to determine the amount of cell surface (L: labeled fraction) and intracellular (UL: unlabeled fraction) content. *P < 0.05 vs. Con, †P < 0.05 vs. labeled fraction.

4. Discussion

Diabetes imposes a considerable medical, economic and social burden on societies and is quickly becoming a worldwide health crisis that requires urgent action. Despite intensive research (primarily in skeletal muscle and adipose tissue), the pathogenic cause of altered glucose transport observed during diabetes remains elusive, and the role of the more newly discovered GLUT12 isoform has not been studied in the diabetic myocardium. Using a progressive biotinylation photolabeling technique uniquely applied to GLUT12 in both the intact perfused heart and isolated myocytes, we demonstrated that

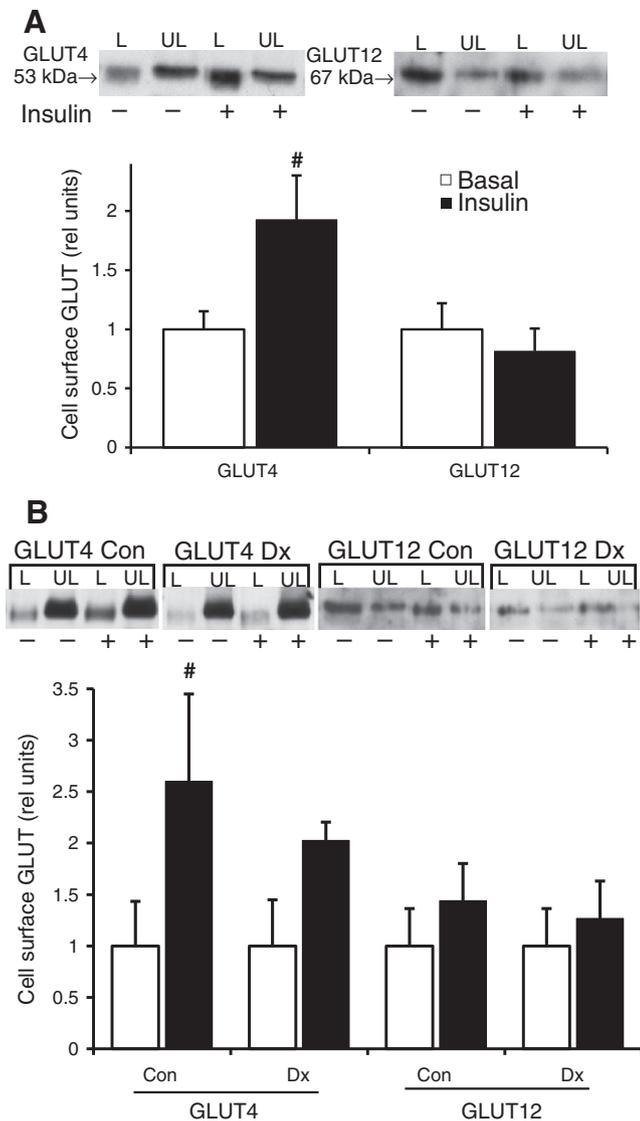


Fig. 3. Insulin stimulation increases cell surface GLUT-4, but not -12, in the heart under physiological conditions. A) Healthy cardiac myocytes were incubated for 20 min without (basal) and with insulin (100 μ M) prior to the cell-surface biotinylation assay. Top panel: Western blot of photolabeled isolated cardiac myocytes. Bottom panel: Mean \pm SE of active cell surface cardiac GLUT protein (expressed relative to basal values; $n=6$ /group). L: labeled fraction (i.e., cell surface pool); UL: unlabeled fraction (i.e., intracellular pool). # $P<0.05$ vs. basal values. B) Diabetes induces alteration in insulin-stimulated GLUT-4, but not -12, translocation in the heart. Isolated intact hearts from healthy (Con) and type 1 diabetic (Dx) mice were perfused with or without insulin (at physiologic concentration, 100 μ M/ml, for 35 min) before photolabeling with bio-LC-ATB-BGPA. Top panel: Western blot of photolabeled myocardium. Bottom panel: Mean \pm SE of active cell surface cardiac GLUT protein (i.e., expressed relative to basal values; $n=4-5$ /group). There is no significant difference in the unlabeled GLUT fraction for any of the groups (with and without insulin stimulation, data not shown). L: labeled fraction (i.e., cell surface pool); UL: unlabeled fraction (i.e., intracellular pool). # $P<0.05$ vs. basal values.

insulin stimulation increased myocardial translocation to the cell surface of GLUT-4, but not -12, under physiological conditions. We further showed that basal active cell surface GLUT12 content was increased in the diabetic myocardium, potentially as a compensatory mechanism for the observed downregulation of active GLUT4, in the face of persistent hyperglycemia. Overall, our data suggest that GLUT12 in the heart functions as a basal GLUT primarily located at the cell surface.

The discovery over 15 years ago that GLUT4 knockout mice do not develop hyperglycemia [5] and still demonstrate modest insulin-stimulated glucose uptake in skeletal muscle [18] clearly suggested

that other GLUT isoforms may play a key role in the regulation of glucose transport. However, data on GLUT12, one of the more newly discovered GLUT isoforms, still remains extremely scarce. While it has been suggested that GLUT12 is primarily located intracellularly in some tissues [9,19] and could be a novel second insulin-sensitive glucose transporter [7,8], the regulation and functional significance of GLUT12 in the heart, one of the main organs to utilize glucose, is unknown. Surprisingly, our data demonstrates that the proportion of GLUT located at the cell surface and intracellularly exhibits starkly opposite regulation for GLUT-4 and -12. Consistent with its well-accepted function as a translocatable GLUT primarily located intracellularly under basal conditions [20], in the present study, the amount of basal active GLUT4 at the cell surface was 31% in the healthy myocardium. Our novel biotinylation results are similar to those obtained by membrane fractionation, suggesting that 28% of basal GLUT4 is membrane-associated in the healthy heart [21]. By contrast, our results show that cell surface GLUT12 contributes to the majority of myocardial GLUT12 protein in the healthy and diabetic state, suggesting that in the heart, GLUT12 functions mainly as a basal cell surface glucose transporter. Furthermore, using both biotinylation and confocal imaging techniques, the present study provides novel evidence that, in contrast to GLUT4, there is no measurable increase in cell surface GLUT12 following physiological insulin stimulation in healthy myocardium, suggesting that insulin-stimulated GLUT12 translocation is not a major regulatory process in the heart. This is contrary to the limited research in skeletal muscle suggesting that GLUT12 translocates to the healthy sarcolemma after insulin stimulation [7,11]. In healthy humans, *in vivo* insulin stimulation increased GLUT12 protein content measured by membrane fractionation [7]. However, it must be noted that many studies investigating GLUT trafficking utilize techniques, which cannot differentiate active GLUTs at the cell surface from inactive GLUTs (i.e., before membrane docking and fusion) [12,15]. Therefore, in the present study, we performed a cell surface biotinylation assay in both the intact perfused hearts and isolated cardiac myocytes, using an impermeable bis-mannose compound, which is specific for GLUTs and only labels those that are accessible (active) at the cell surface. Complementary to the confirmatory confocal imaging analysis performed, this biotinylation technique allows us to reliably quantify the proportion of active GLUT at the myocardial cell surface, and to our knowledge we are the first to successfully apply this methodology to GLUT12.

Overall, it appears that GLUT12 function and regulation is different in the heart compared with other insulin-sensitive tissues (i.e., skeletal muscle and adipose). Interestingly, our findings are consistent with and further build upon a recent study performed in transgenic mice overexpressing GLUT12 [8]. Although this study demonstrated increased whole body glucose tolerance and enhanced insulin-stimulated (but not basal) glucose uptake in insulin-sensitive tissues of transgenic mice overexpressing GLUT12, the enhanced glucose clearance with GLUT12 overexpression showed tissue specificity: In the heart, insulin stimulation resulted in a 140 and 144.5% increase in glucose clearance compared with basal conditions in wild type and transgenic mice, respectively. By contrast, the increase in insulin stimulated glucose clearance was markedly higher in all other peripheral tissues studied (an increase of 1090 and 2380% in EDL, 600 and 1200% in soleus, and 150 and 290% in fat, was reported in wildtype and transgenic mice, respectively) [8]. These previous findings are especially interesting in light of the ~2-fold higher degree of GLUT12 protein overexpression in the heart compared to all other tissues studied [8]. In addition, insulin also does not stimulate acute translocation of GLUT12 in MCF-7 cancer cells [9]. Taken together, the present and previous studies provide new evidence that, while GLUT12 contributes to whole body insulin sensitivity and may function as a second insulin-regulatable transporter in skeletal muscle and adipose tissue, its primary role in the heart appears to be as a basal non-translocatable GLUT associated with the cell surface. While a cause for the discrepancy

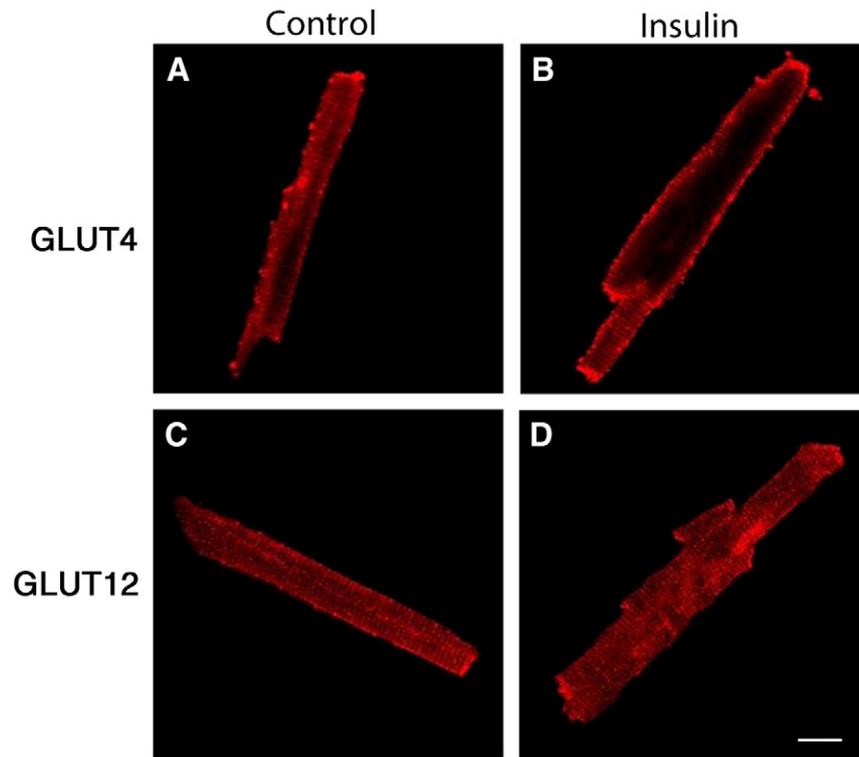


Fig. 4. Insulin stimulation causes significant translocation of GLUT4, but not GLUT12, to cell peripherals, as visualized by confocal laser scanning microscopy. Adult rat cardiac myocytes were incubated without (A, C) or with insulin (B, D, 100 μ U/ml) for 30 min prior to immunofluorescent staining. Results were analyzed from three independent experiments with at least 70 cells/treatment. Scale bar: 10 μ m.

between cardiac and skeletal muscle insulin-mediated regulation is not readily apparent, a similar finding has been reported for another GLUT isoform, GLUT1. GLUT1 has been referred to as another basal transporter in the heart (known to be primarily associated with the plasma membrane and to exhibit minimal insulin-stimulated translocation) [21,24], while it has been shown to translocate to the adipocyte plasma membrane upon insulin stimulation [25]. Therefore, such discrepancies may reflect tissue specific regulation of GLUTs. In addition, since contraction-induced GLUT4 translocation could be a major mechanism regulating cardiac glucose transport, further studies are required to investigate the potential role of calcium/contraction pathways on the regulation of GLUT12 in the heart [1].

Although there is a continued lack of understanding of the pathogenic mechanisms underlying diabetes and its cardiac complications, several studies have investigated the pathogenic role of GLUT4 [1]. In the present, we used a type 1 diabetic model in order to best investigate insulin-stimulated GLUT12 regulation, since the confounding effect of endogenous insulin is down-regulated to null. This animal model is also highly relevant to type 2 (non-insulin dependent) diabetes. In particular, streptozotocin-induced type 1 diabetes also results in peripheral insulin resistance and, in particular, impaired cardiac insulin signaling and GLUT4 translocation [22,23]. Furthermore, while there is initially insulin resistance in type 2 diabetes, as the disease progresses there is also insulin deficiency secondary to the exhaustion of pancreatic beta cells. Consistent with previous reports using membrane fractionation of the heart [26,27], we observed a decreased cell surface GLUT4 content in both the intact perfused heart and isolated myocytes in our diabetic model. In addition, we also observed impairment in insulin-stimulated GLUT4 translocation, which reflects the fact that type 1 diabetes also results in peripheral insulin resistance and impaired cardiac insulin signaling [22,23]. Similarly, GLUT12 could be a major player in the pathogenesis of altered glucose transport during insulin resistance and diabetes; however, its role during these pathophysiological states, especially in the heart,

remains unclear. Our results demonstrated a 2.5 fold increase in basal cell surface GLUT12 content in the diabetic heart, which was an inverse response to that of GLUT4. By contrast, the limited literature focusing on other insulin-sensitive tissues has not shown any differences in GLUT12, either in skeletal muscle mRNA expression from human type II diabetics [28], or in protein expression of skeletal muscle and adipose tissue in a large animal model of naturally-occurring compensated insulin resistance [11,12]. However it should be noted that, in contrast to the type 1 diabetic model employed in the present study, the insulin resistant/diabetic subjects from these previous studies did not exhibit significant hyperglycemia [11,12,28]. Therefore, although the mechanisms inducing this apparent upregulation in primarily basal GLUTs (e.g., GLUT12) is not well-elucidated, they broadly appear to be a consequence of the systemic disturbances in glucose metabolism [1,29].

Since the heart is one of the main organs to utilize glucose [1], we investigated the relationship between active cell surface cardiac GLUTs and whole-body glucose homeostasis. It is well established that altering cell surface GLUT4 content in insulin-sensitive tissues affects peripheral blood [glucose] [4–6], and cell surface GLUT4 content of skeletal muscle and glucose transport are known to be closely associated in the healthy and diabetic state [30,31]. The present data provides further evidence that cardiac GLUT4 plays a role in regulating whole body glucose homeostasis, such that active cell surface GLUT4 was negatively correlated with blood [glucose] in healthy and diabetic mice. By contrast, we demonstrated a significant positive correlation between active cell surface GLUT12 and blood [glucose] in the healthy and diabetic heart, suggesting that a compensatory increase in the basal GLUT12 could occur secondary to impaired insulin-mediated GLUT4 signaling and concomitant dysregulation of glucose metabolism. Indeed, certain pathophysiological states known to decrease cardiac GLUT4 content such as cardiac ischemia and heart failure have concomitantly been shown to result in increased basal GLUT1 [1,32,33] and GLUT12 [10]. Type 1 diabetes

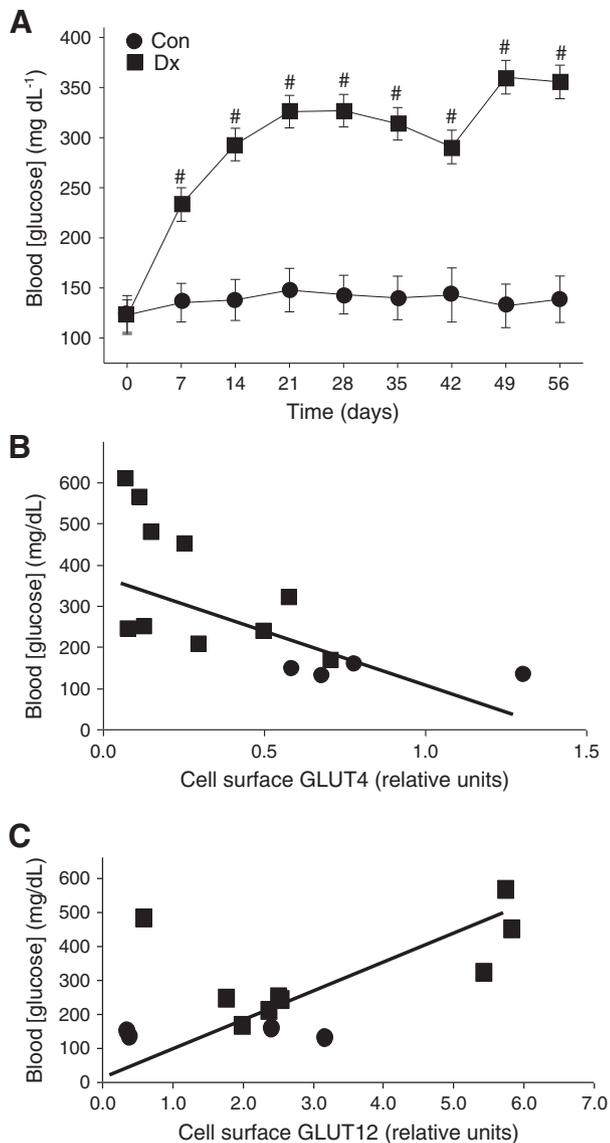


Fig. 5. Scatterplot and linear regressions of myocardial cell surface GLUT content (independent variable) and venous blood [glucose] (dependent variable) show significant correlations between active cell surface GLUT and blood [glucose] in the healthy and diabetic myocardium. A) Type 1 diabetes results in sustained hyperglycemia. Mean \pm SE venous fasting blood glucose concentration in healthy (Con) and diabetic (Dx) mice obtained at baseline (0) and up to 56 days after final streptozotocin injection ($n = 7$ –10/group), # $P < 0.05$ vs. baseline (0). B) In vivo blood [glucose] was negatively correlated with active basal cell surface GLUT4 in the healthy and diabetic myocardium at 8 weeks post induction of diabetes ($n = 4$ –10/group). $P = 0.007$, $R_{sq} = 0.463$. [glucose] = $433.724 - (316.792 \times GLUT4)$. C) By contrast, blood [glucose] was positively correlated with active basal cell surface GLUT12 in the healthy and diabetic myocardium ($n = 4$ –10/group). $P = 0.045$, $R_{sq} = 0.317$. [glucose] = $153.244 + (42.349 \times GLUT12)$.

also results in the same inverse changes between GLUT-4 and -1 in skeletal muscle [29], and presently we further demonstrate similar findings with GLUT-4 and -12 in cardiac muscle. Taken together, these results suggest that the functional effect of cardiac GLUT12 upregulation is of minor consequence to overall glucose metabolism. However, further studies will be required to establish if the observed GLUT12 upregulation is an adaptive response with at least some functional and beneficial consequence for preserving myocardial glucose transport. In addition, further studies will be required to evaluate the functional significance of GLUT12 in comparison with other established basal GLUTs (e.g., GLUT-1 and -5). Indeed, given the prevalence of associated cardiovascular co-morbidities and lack of effective insulin sensitizing therapy for diabetic patients, the non-insulin

stimulated GLUTs represent an intriguing therapeutic avenue. For example, while research has shown that exercise training during diabetes has limited benefit to restore cardiac GLUT4 content [27], results in healthy human skeletal muscle suggest that exercise-induced increases in GLUT content, which is at least 2-fold greater for GLUT12 than any of the other isoforms studied including GLUT4 [34]. Thus further investigation into the regulation of the class III of GLUTs is still needed, particularly with respect to their overall contribution to cardiac glucose uptake in healthy and pathophysiological states.

5. Conclusions

In summary, using a cell surface biotinylation assay in both the intact perfused hearts and isolated cardiac myocytes as well as immunofluorescent confocal microscopy, we demonstrated that physiologic insulin-stimulation does not significantly regulate myocardial GLUT12 translocation to the cell surface. In addition, active cell surface GLUT12 was increased in the diabetic myocardium, potentially as a compensatory mechanism for the observed downregulation of active GLUT4. Collectively our data suggest that, in contrast to GLUT4, GLUT12 appears to function as a basal GLUT located primarily at the cell surface in the heart.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbadis.2012.09.013>.

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